A renaissance in medical biochemistry – Hepatology and Endocrinology kick it up a Notch!

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Abstract

Transcription factor FoxO1 promotes hepatic glucose production. Genetic inhibition of FoxO1 function prevents diabetes in experimental animal models, providing impetus to identify pharmacological approaches to modulate this function. Altered Notch signaling is evident in tumorigenesis, and Notch antagonists are in clinical testing for application in cancer. Here we report that FoxO1 and Notch coordinately regulate hepatic glucose metabolism. Combined haploinsufficiency of FoxO1 and Notch1 markedly raises insulin sensitivity in diet-induced insulin resistance, as does liver-specific knockout of the Notch transcriptional effector Rbp-J

Conversely, Notch1 gain-of-function promotes insulin resistance in a FoxO1-dependent manner and induces glucose-6-phosphatase expression. Pharmacological blockade of Notch signaling with γ-secretase inhibitors raises insulin sensitivity after in vivo administration in lean mice and in obese, insulin-resistant mice. The data identify a heretofore unknown metabolic function of Notch and suggest that Notch inhibition is beneficial in diabetes treatment, in part by helping to offset excessive FoxO1-driven hepatic glucose production.

AND


Considerable data support the idea that forkhead box O1 (Foxo1) drives the liver transcriptional program during fasting and is then inhibited by thymoma viral proto-oncogene 1 (Akt) after feeding. Here we show that mice with hepatic deletion of Akt1 and Akt2 were glucose intolerant, insulin resistant and defective in their transcriptional response to feeding in the liver. These defects were normalized with concomitant liver-specific deletion of Foxo1. Notably, in the absence of both Akt and Foxo1, mice adapted appropriately to both the fasted and fed state, and insulin suppressed hepatic glucose production normally. A gene expression analysis revealed that deletion of Akt in liver led to the constitutive activation of Foxo1-dependent gene expression, but

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Conflict of interest The author declared that he does not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.
again, concomitant ablation of FoxO1 restored postprandial regulation, preventing the inhibition of the metabolic response to nutrient intake caused by deletion of Akt. These results are inconsistent with the canonical model of hepatic metabolism in which Akt is an obligate intermediate for proper insulin signaling. Rather, they show that a major role of hepatic Akt is to restrain the activity of FoxO1 and that in the absence of FoxO1, Akt is largely dispensable for insulin- and nutrient-mediated hepatic metabolic regulation in vivo.

**Keywords**

Notch; Insulin resistance; Liver metabolism; Akt

Today, as I pen this editorial, the Institute of Medicine Committee on Accelerating Progress in Obesity Prevention of the United States releases a report projecting higher rates of obesity in children as well as adult men and women in the US in the not too distant future [1]. The initial text of the first of two articles—both from *Nature Medicine*—underscores the pressing concerns of the obesity epidemic: “TYPE 2 diabetes is associated with obesity and insulin resistance” [2], while the second declares “In mammals, the obligate requirement for at least some simple carbohydrate at all times is fulfilled during fasting by the liver ...” [3]. As I see it, these articles reflect the growing interface between Endocrinology and Hepatology rooted in modern medical biochemistry, to put an end to insulin resistance.

Both articles focus on FoxO1, the forkhead box-containing transcription factor that is a downstream target in the insulin signaling pathway. FoxO1, in the presence of normal insulin signal transduction, is ultimately inactivated just upstream by phosphorylated Akt (pAkt). While both molecules are key regulating proteins in the insulin signal transduction pathway, the bottom line is, hepatic Akt2 phosphorylation of FoxO1 shuts off FoxO1’s role in the transcriptional activation of genes associated with gluconeogenesis. For different reasons, both reports are explicit that FoxO1 is a poor target for drug development; and *FoxO1* knockout experiments both *in vivo* and *in vitro* in all but rodent models of insulin resistance (insulin receptor knockout mice) offer surprisingly little improvement in suppressing hepatic glucose output, i.e., does little to improve insulin resistance [4]. Using different hypotheses and elegant approaches, both manuscripts provide intriguing and robust data that upend the insulin signaling pathway-Akt–FoxO1 axis; and instead provide novel evidence for new partners that could inhibit transcription of gluconeogenic genes by FoxO1, under conditions of insulin resistance.

In the manuscript by Pajvani et al. [2], the authors demonstrate that FoxO1 and the transcription factor Rpb-J have a physical relationship, which activates Notch target genes. One would not likely make the association between Notch, Jagged, and the Hes-related families of genes with FoxO1, but in doing so, Pajavani’s group reasons that there is a mechanistic relationship between the PI3K–Akt–FoxO1 portion of the insulin signaling cascade and the Notch–Rbp-J signaling apparatus—a cascade usually involved in development and neoplasia. As is standard for Nature papers, there are a plethora of elegant experiments to review; and, it is sometimes difficult to figure out whether the mice are on a high fat diet or a standard one.

By creating *FoxO1*+/− and *Notch1*+/− mice, and cross-breeding them (*FoxO1*+/−; *Notch1*+/−) the investigators demonstrate there is stepwise improvement in insulin sensitivity over wild-type mice (suppressing hepatic glucose production) in both *FoxO1*+/− and *FoxO1*+/−; *Notch1*+/− mice; however, insulin sensitivity is more robust *in FoxO1*+/−; *Notch1*+/− than in *FoxO1*+/− alone. The authors also provide compelling data that Notch1 itself has a critical role to play, at least *in vitro*, because Rbp-J appears to act as a transcriptional activator of...
glucose-6-phosphatase, a key enzyme that initiates glycolysis, in the fasted state, and by employing gain-of-function studies with respective adenoviral constructs, both FoxO1 and Notch1 delivery induces glucose-6-phosphatase without affecting other FoxO1 targets or FoxO1 phosphorylation. Indeed FoxO1<sup>+/−</sup>; Notch1<sup>+/−</sup> mice show a 35% decrease in fasting glucose-6-phosphatase expression and a decrease of nearly 20% of glucose levels. Nonetheless, the authors make a significant case that both FoxO1 and Notch1 play key roles in the full expression of diet-induced hepatic insulin resistance.

In the second publication, Lu and colleagues created liver specific knockout mice for either Akt-1 or Akt2, or both Akt1 and Akt2, which were termed DLKO [3]. After initially demonstrating Akt1 can serve as a ‘proxy’ for hepatic Akt2 in its absence, experiments conducted with standard chow resulted in ‘virtually undetectable’ phosphorylation of Akt from DLKO livers. Yet, in spite of the status of this observation, the investigators observed normal phosphorylation of glycogen synthase kinase 3 (Gsk3α and Gsk3β). Interestingly, even though FoxO1 is a major target of activated Akt, and the insulin receptor substrate 2 (Irs2) a direct Foxo1 target gene [5], the authors found Irs2 to be significantly higher in the DLKO mice compared to control mice created for Akt1 and Akt2. As anticipated, the authors exhaustively demonstrated that the DLKO livers were terribly insulin resistant; but they reasoned that if a promiscuous FoxO1 was the linchpin for this problem, ablating it from mouse liver should make things significantly better. Amazingly, the triple liver specific knockouts (TLKO, Akt1/Akt2/FoxO1) abolished insulin resistance. In performing numerous additional experiments, including genome wide association screening of the DLKO and TLKO mice, the authors came to the stunning conclusion—which sharply challenges the prevailing paradigm—that Akt-dependent inhibition of FoxO1 was not the only arbiter of hepatic glucose homeostasis.

Before I get too far afield from Hepatology, both papers elegantly examine carbohydrate metabolism, but did not study the lipogenic pathways—also controlled by insulin signal transduction—and whether or how hepatic triglyceride stores are affected. Nonetheless, in identifying Notch1 as a regulator of glucose metabolism in the first manuscript, and the second implicating an insulin-dependent, Akt-independent pathway for suppressing liver glucose output, the Endocrinologist can go after Notch1 or another, as yet unidentified, molecule(s) to reverse hyperglycemia and hepatic insulin resistance in type 2 diabetes mellitus. Fig. 1 highlights the important lessons to take away from these two excellent articles. The question for us as Hepatologists, then, is whether these novel discoveries are equally important in hepatic triglyceride metabolism, because if these findings are truly relevant to our patients, we need to prove it and target such molecules accordingly!

**Acknowledgments**

**Financial support** This work was supported by United States Public Health Service (USPHS) R01DK062092 and USDOVAI01BX001746, and by a grant from the University Research Council of Emory University.

The underlying research reported in the study was funded by the US Public Health Service (USPHS) National Institutes’ of Health (NIH).

**References**


Fig. 1. Pathways of hepatic glucose metabolism: old and new
(A) Identity of key players in the hepatocyte insulin signaling pathway. In the fasted state, the forkhead protein FoxO1 binds the promoters of key genes associated with gluconeogenesis, and glycolysis (phosphoenolpyruvate carboxykinase [PEPCK] and glucose-6-phosphatase [G6P], respectively. (B) In the fed state, when insulin secretion is increased and glucose is plentiful, the insulin signaling pathway is activated via phosphorylation of the elements identified in (A). In the absence of insulin resistance, phosphorylation of FoxO1 heralds an end to its ability to activate transcription of PEPCK, G6P, and other genes associated with glycogen breakdown and glucose synthesis. (C) The TLKO mice studies by Lu and colleagues strongly contends that another pathway serves to shut off glucose production even when Foxo1 is not phosphorylated (as is thought to be the case in the insulin resistance state). (D) Pajvani’s group contends, on the other hand, that the Notch1-Jagged pathway in the state of insulin resistance syndrome activates the downstream transcription factor Rbp-Jk, which either with FoxO1, or independently can activated G6P and thereby enhance hepatic glucose production. These data imply that Notch1 may be an important target in combating insulin resistance because it is easier to therapeutically target than FoxO1.